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MTHFR polymorphisms in relation to ovarian cancer risk

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Abstract

Objective—Folate has been hypothesized to influence carcinogenesis due to its dual role in DNA methylation, which regulates gene expression, and synthesis of purine and thymidylate, which is vital for DNA repair. Thus, we examined ovarian cancer risk in relation to two functional polymorphisms (C677T and A1298C) in the *MTHFR* gene.

Methods—We genotyped the C677T (rs1801133) and A1298C (rs1801131) *MTHFR* polymorphisms in 1642 cases and 2068 controls from three studies, the New England Case Control Study (NEC), Nurses' Health Study (NHS), and Mayo Clinic Ovarian Cancer Case Control Study (MAY).

Results—Overall, we observed no association between either SNP and ovarian cancer risk (pooled C677T p_{trend} = 0.59 and A1298C p_{trend} = 0.58). Significant associations (C677T p_{trend}=0.001, A1298C $p_{trend=}$0.02) between these *MTHFR* SNPs and serous ovarian cancer risk were observed in the NEC study, but were not replicated in the NHS and MAY studies.

Conclusions—*MTHFR* SNPs C677T and A1298C are not associated with ovarian cancer risk. Our results highlight the need for validation of genetic findings.

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INTRODUCTION

Folate has been hypothesized to contribute to carcinogenesis because of its dual role in DNA methylation, which can modulate expression of oncogenes, and synthesis of purine and thymidylate, which is vital for DNA repair. Low folate intake has been associated with an increased risk of several cancers, including breast, colon, and endometrial cancer. Its influence on ovarian cancer risk is less clear and may depend on other unmeasured factors such as genetic variation in the folate metabolism pathway. 5,10-methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of folate from 5,10-methylenetetrahydrofolate (5,10-MTHF), which is needed for DNA synthesis, to 5-methyltetrahydrofolate (5-MTHF), which is used in the methylation of homocysteine to form methionine. Two *MTHFR* polymorphisms (C677T and A1298C) have been associated with reduced enzyme activity, leading to an increase in 5,10-MTHF and a decrease in 5-MTHF. These polymorphisms have been associated with risk of a variety of cancers, including colon, prostate, endometrial, and breast. Here we examine the role of these two functional polymorphisms in relation to ovarian cancer risk in three study populations.

MATERIALS AND METHODS

Study population

New England Case-Control Study (NEC)—Data and specimens from the New England-based case-control study of ovarian cancer come from two enrollment phases (1992–1997 and 1998–2002). Briefly, women residing in eastern Massachusetts or New Hampshire with a diagnosis of incident ovarian cancer were identified through hospital tumor boards and statewide cancer registries. 71% of the eligible cases agreed to participate. Controls were identified through a combination of random digit dialing, drivers' license lists, and town resident lists. In the first phase, 72% of the eligible women identified through random digit dialing agreed to participate and 51% of the eligible women identified through townbooks agreed to participate. In the second phase, 57% of eligible controls were enrolled. Controls were frequency matched to cases on age and state of residence.

All study participants were interviewed at the time of enrollment about known and suspected ovarian cancer risk factors. Over 95% of the participants provided a blood specimen. All cases were followed up for survival. Death dates were ascertained through the National Death Index Plus and hospital cancer registries.

Nurses' Health Study (NHS)—The NHS cohort was established in 1976 among 121,700 U.S. female registered nurses, ages 30 to 55 years. Women completed an initial questionnaire and have been followed biennially by questionnaire to update exposure status and disease diagnoses. In 1989–90, 32,826 participants submitted a blood sample; details of the collection are described elsewhere. Follow-up of the NHS blood study cohort was 98% in 2004. We identified new diagnoses of ovarian cancer in several ways. All self-reported diagnoses and deaths reported by family members or identified through the U.S. National Death Index or U.S. Postal Service were followed up for confirmation. The nurse's family was contacted to request permission to obtain medical records related to the diagnosis, particularly pathology reports. Approximately 98% of deaths within NHS are captured by the U.S. National Death Index. For this analysis, we included all epithelial cases who submitted a blood sample prior to diagnosis or within 4 years after diagnosis. All cases were diagnosed before June 1, 2004 and had no history of a prior cancer, other than non-melanoma skin cancer.

We randomly selected three controls per case from the study participants with DNA available, no prior bilateral oophorectomy, and no history of cancer, other than non-melanoma skin cancer, at the questionnaire cycle of diagnosis of the case and matched on age, menopausal

status at baseline and diagnosis, month of blood collection, time of day of blood draw, fasting status, and postmenopausal hormone use at blood draw.

Mayo Clinic Ovarian Cancer Case Control Study (MAY)—This clinic-based case-control study began in 2000 at the Mayo Clinic in Rochester, MN; details of the study design have been described previously. Briefly, cases were women more than 20 years of age with histologically-confirmed incident epithelial ovarian cancer and residing in six states that comprise the primary catchment area for the Mayo Clinic. Controls were women with at least one intact ovary and no history of ovarian cancer seeking general medical care from the outpatient primary care practice at Mayo Clinic. Controls were matched in 5-year age categories and region of residence to cases. Of those who were invited, 89% of cases and 80% of controls agreed to participate. Data on known and suspected risk factors for ovarian cancer were collected through in-person interviews. Participants donated an extra tube of blood during regularly scheduled medical care, including cases, who had blood drawn before initiating chemotherapy.

All three studies obtained IRB approval from their respective institutions and informed consent from all participants.

Genotyping Methods

DNA was extracted from NEC and NHS buffy coat samples using QIAmp (Qiagen, Chatsworth, CA); due to limited availability of genomic DNA, samples were amplified using Genomiphi (GE Healthcare, Piscataway, NJ). For NEC and NHS samples, genotyping of *MTHFR* SNPs C677T (rs1801133) and A1298C (rs1801131) was performed at the Dana Farber Harvard Cancer Center High Throughput Polymorphism Detection Core. Genotyping assays were performed by the 5′ nuclease assay (Taqman®) on the Applied Biosystems Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, California). Taqman ® primers, probes, and conditions for genotyping assays are available upon request. Replicate samples (approximately 10%) were included for quality control. Genotyping was performed by laboratory personnel blinded to case-control status and quality control replicates. Over 95% of the samples were successfully genotyped for each polymorphism. Concordance between replicate samples was 100%.

For MAY samples, genomic DNA was extracted from blood using Gentra AutoPure LS Purgene (Qiagen, Valencia, CA) salting out method. Genotyping was performed at the Mayo Clinic Genotyping Shared Resource using the Illumina GoldenGate BeadArray assay and BeadStudio software for automated genotype clustering and calling, as described previously. Quality control included three standard DNA samples and two duplicates on each plate as well as CEPH family trios from the Coriell Institute on eight plates, summing to 74 quality control samples or 8% of the total sample size. Both SNPs had greater than 95% genotyping success and 99.99% concordance between duplicate samples.

Statistical analysis

Analyses were restricted to Caucasian study participants because allele frequencies varied by race. We used chi-square tests to assess Hardy-Weinberg Equilibrium (HWE) for each SNP among controls.

We calculated odds ratios (OR) and 95% confidence intervals (95% CI) using unconditional logistic regression (NEC, MAY), adjusting for matching factors, or conditional logistic regression (NHS). In covariate-adjusted models, we additionally controlled for oral contraceptive use, parity, and tubal ligation (tubal ligation data were not available for the MAY study).

We compared women carrying one (heterozygous) or two (homozygous variant) copies of the minor allele to women carrying two common alleles (wild type). Trend tests also were calculated using a variable representing the number of variant alleles (0, 1, or 2). We calculated mortality rate ratios using Cox proportional hazards models, with time since diagnosis as the time-scale, in order to assess whether polymorphisms were associated with survival in the NEC study. Pooled odds ratios were estimated using meta-analyses with inverse-variance weighting. All analyses were performed using SAS v 9.1 (SAS, Cary, North Carolina) and Intercooled Stata 9 (StataCorp LP, College Station, Texas).

RESULTS

We assessed the association of *MTHFR* SNPs with ovarian cancer risk in 1642 cases and 2068 controls from three study populations (1120 cases and 1160 controls from NEC, 158 cases and 496 controls from NHS, 364 cases and 412 controls from MAY). Compared with controls, cases were less likely to have taken oral contraceptives or have had a tubal ligation but were more likely to be nulliparous (Table 1). The distribution of tumor histology and behavior modestly differed by study. Compared to NHS and MAY, the NEC study had more mucinous and clear cell cases while MAY had more serous and endometrioid cases than the other two studies. Invasive tumors accounted for 85% of the cases in NHS and MAY compared to 76% in NEC.

Minor allele frequencies (MAF) in controls were similar across the NEC, NHS and MAY studies for *MTHFR* C677T (MAF = 0.28, 0.28, 0.27, respectively) and A1298C (MAF = 0.26, 0.26, 0.27, respectively). Overall, we observed no significant association between the *MTHFR* polymorphisms and ovarian cancer risk (Table 2) and no significant heterogeneity between studies when all cases were considered. For the C677T SNP, the pooled odds ratios were 1.16 (95% CI = 1.00, 1.35) for the CT genotype and 0.95 (95% CI=0.64, 1.40) for TT genotype. For the A1298C SNP, the pooled odds ratios were 0.97 (95% CI=0.84, 1.12) for the AC genotype and 0.96 (95% CI=0.75, 1.24) for the CC genotype. When we restricted to serous invasive cases, we observed significant heterogeneity in results for the C677T homozygous variants across the three studies (p=0.01). In the NEC study, we observed an increased risk of serous invasive ovarian cancer with the C677T variant (p_{trend}= 0.001), but no association in NHS (p_{trend}= 0.16) or MAY (p_{trend}= 0.86). Similarly, the decreased risk of serous invasive ovarian cancer with the A1298C variant (p_{trend}= 0.02) that we observed in NEC was not replicated in NHS (p_{trend}= 0.81) or MAY (p_{trend}= 0.75) although there was no significant heterogeneity between estimates ($p_{heterogeneity}$=0.19). In the NHS study, restricting to the 141 incident cases did not substantively change the results (data not shown). We observed no interaction between the *MTHFR* SNPs and parity, tubal ligation, oral contraceptive use, Jewish religion, or age (data not shown). Interestingly, among NEC cases, the prevalence of the C677T variant was higher among women who had received chemotherapy (383 (62%) 677T carriers among cases who received chemotherapy vs. 249 (56%) 677T carriers among cases who had not received chemotherapy, p=0.05) or a transfusion (212 (64%) 677T carriers among cases who had a transfusion vs. 415 (58%) among cases who had not received a transfusion, p=0.06).

Among NEC cases, we observed 453 deaths (431 deaths among invasive cases) with a mean follow up time of 8.7 years (mean follow up time for invasive cases = 7.7 years). Among invasive cases, we observed no statistically significant association between C677T and ovarian cancer survival (Hazard Ratio $(HR)_{TT\ vs.\ CC}$ = 1.16, 95% CI = 0.87–1.56) or A1298C ($HR_{CC\ vs.\ AA}$= 1.01, 95% CI= 0.70, 1.45). These associations did not change when we restricted to serous invasive cases, borderline cases, cases treated with chemotherapy, or cases enrolled pre-operatively, although numbers of cases were small (data not shown).

DISCUSSION

In this study, we evaluated *MTHFR* polymorphisms important to DNA synthesis and methylation. The association between *MTHFR* polymorphisms C677T (rs1801133) and A2198C (rs1801131) and ovarian cancer risk was inconsistent between studies. We observed significant associations with serous invasive ovarian cancer for women with homozygous variant genotypes in the NEC, but no association in the NHS and MAY studies.

Biologic studies have demonstrated the functional significance of the C677T polymorphism. Compared to wild type, heterozygotes have a 65% lower enzyme activity while homozygous variants (TT) have a 30% lower activity. Furthermore, the TT genotype is associated with lower plasma folate, higher homocysteine levels, and lower DNA methylation levels in vivo. Like the C677T variant, the A1298C variant also has been reported to reduce enzyme activity. However, the data regarding A1298C and plasma folate or homocysteine levels are less consistent.

Epidemiologic studies of genetic polymorphisms have been plagued by inconsistent results. Study differences in race and ethnicity (population stratification) are often cited as a possible explanation for conflicting conclusions. To address potential differences in ancestry, we restricted the analysis to Caucasian women. Wacholder and colleagues reported that residual confounding after restricting to Caucasians is likely to be minimal (<1%); therefore, any bias due to population differences is small. In addition, we observed similar allele frequencies for controls across all three studies, suggesting that our study populations were comparable. However, allele distributions in the cases varied widely between studies, leading to the differences in associations across populations. These may be due to differences in the way the cases were collected in the three studies. The New England Case-Control Study is a population based study that identified cases through hospital tumor boards and cancer registries; the mean time between diagnosis and study entry was 9.9 months. The Mayo study is clinic-based; cases were identified quickly and enrolled before chemotherapy. The higher proportion of invasive cases in the MAY study (85%) compared to the NEC study (76%) suggests that this approach allowed investigators to enroll more aggressive cases before they became too sick to participate. Alternatively, the higher proportion of invasive cases could reflect different base populations. Specifically, more aggressive cases may be referred to a tertiary care center like the Mayo Clinic than cases identified in the general population; although matching of controls by geographic region should minimize potential confounding within the MAY study. The Nurses' Health Study is a prospective cohort in which women were enrolled before the development of disease and followed prospectively. If the polymorphisms under study impart a survival advantage, then we would expect to observe an excess of the variant alleles among cases with a greater enrollment delay after diagnosis since they had greater opportunity to die before study entry. Consequently, we would expect the strongest associations in a population-based study design, attenuated associations in a clinic-based study design, and no association in a prospective cohort, which is consistent with our data. However, neither the NEC survival analysis (reported here) nor a previous MAY survival analysis reported an association between these *MTHFR* SNPs and ovarian cancer prognosis. Lack of a survival association may be due to limited sample size (MAY) or loss of cases before enrollment (NEC). Alternatively, we may have observed conflicting associations due to chance.

Interestingly, some biologic data support a potential for survival bias for the *MTHFR* SNPs. The balance of the 5,10-MTHF and 5-MTHF, which is controlled by MTHFR, may influence the efficacy of treatment with 5-fluorouracil (5-FU) chemotherapy. Polymorphisms that decrease the activity of MTHFR will lead to increased amounts of 5,10-MTHF. Cell line experiments suggest that fluoropyrimidines like 5-FU exert their effect by inhibiting thymidylate synthetase through the formation of a ternary complex, involving 5-FU,

thymidylate synthetase, and 5,10-MTHF. Therefore, these *MTHFR* polymorphisms may enhance the effect of 5-FU by increasing the amount of 5,10-MTHF available for the complex. In animal models, the administration of 5,10-MTHF led to enhanced efficacy of 5-FU. Further, Cohen and colleagues observed that among colon cancer cases who received 5-FU, those carrying the *MTHFR* C677T variant were nearly three times more likely to respond to therapy compared to those with the wild type genotype. A reduction in growth of colon and lung cancer cell lines with the dual administration of MTHFR antisense and 5-FU or cisplatin suggests the *MTHFR* polymorphism may also be relevant to cisplatin therapy. Whether the *MTHFR* polymorphism and chemotherapy interaction can be extended to ovarian cancer is unclear. Although we observed more cases with the C677T variant than expected among those receiving chemotherapy, we did not observe a survival advantage for cases with the C677T, after accounting for chemotherapy history, although the number of cases in each group was small.

Three previous studies have examined the association between the *MTHFR* polymorphisms and ovarian cancer risk. Jakubowska and colleagues examined both the C677T and A1298C polymorphisms in relation to ovarian cancer among Polish *BRCA1* carriers (146 cases, 280 controls) identified through a cancer registry. They observed an increased risk of ovarian cancer for women who were homozygous for the C677T variant (OR=1.64, 95% CI=1.15, 2.34) and a non-significant reduction in risk for women with two 1298C alleles (OR=0.83, 95% CI=0.47, 1.48). Gershoni-Baruch and colleagues assessed the role of the C677T polymorphism among Jewish women with either breast or ovarian cancer. They observed that the 677T allele was not associated with breast or ovarian cancer alone but was associated with risk of bilateral breast or combined breast and ovarian cancers. Although *BRCA1/2* status is not available for NEC study participants, we observed no association between the *MTHFR* polymorphisms and ovarian cancer among Jewish participants (96 cases, 64 controls). Kelemen and colleagues reported no association between 16 *MTHFR* polymorphisms and ovarian cancer among 829 ovarian cancer cases and 941 controls of known or presumed Caucasian ancestry from Duke University and Mayo Clinic as part of a larger analysis of 188 SNPs in the one-carbon transfer pathway. Specifically, the combined Duke/Mayo results for rs1801131 ($OR_{ACvs.AA}$=0.9, 95% CI=0.7–1.1; $OR_{CCvs.AA}$=1.0, 95% CI=0.7–1.4) and rs1801133 ($OR_{CTvs.CC}$=1.1, 95% CI=0.9–1.3; $OR_{TTvs.CC}$=0.9, 95% CI=0.6–1.2) are similar to the results presented here for known Caucasians in the MAY study. Since the Duke study, like NEC, has a population based design, these null results suggest study design cannot entirely account for the discordance we observed between studies.

Two recent genome-wide association studies (GWAS) of ovarian cancer show no association between A1298C (rs1801131) and ovarian cancer but results for the C677T (rs1801133) SNP are mixed. The US-based GWAS, which includes participants from multiple centers including the NEC and MAY studies, showed an increased risk with the rs1801133 variant that remained significant after restricting to 2836 participants not in the NEC or MAY studies (OR=1.16, p=0.01) (personal communication Y. Tsai, July 14, 2010). Yet, unpublished findings from the UK-based GWAS show the same SNP was not associated with overall ovarian cancer risk (OR=0.96, p=0.32) based on 4258 participants. Again differences in population characteristics, including enrichment of hereditary cases in the UK GWAS, and study design likely explain the discrepant results.

Strengths of our study include its large sample size and use of three distinct study populations. Comparison of associations and allele distributions across study populations, particularly the inclusion of a prospective study, allowed us to identify potential sources of bias. Overwhelmingly, case-control studies are useful in the evaluation of genetic susceptibility to ovarian cancer since this disease is rare and genotypes assessed in genomic DNA do not change with altered disease status. However, when genetic variants impart a survival advantage, collection after diagnosis invites the possibility of survival bias. Our study highlights the need

for comparison of allele frequencies in independent studies of varying design and the possible role of chance in disparate results across study populations.

In conclusion, we observed that functional polymorphisms (C677T, A1298C) in a key folate metabolism enzyme (MTHFR) were not associated with ovarian cancer risk. A broader evaluation of this association is warranted addressing additional genetic variations in the folate metabolism pathway using a larger study population with prospectively collected samples to minimize the role of chance and influence of a possible survival advantage for those treated with chemotherapy.

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Table 1

Characteristics of Caucasian study participants in the New England Case Control Study (NEC), Nurses' Health Study (NHS), and Mayo Clinic Ovarian Cancer Case Control Study (MAY)

Characteristic	NEC			NHS			MAY		
	Cases	Controls	p	Cases	Controls	p	Cases	Controls	p
n	1120	1160		158	496		364	412	
Age, yrs [mean, (sd)]	52 (13)	51 (13)	0.43	61 (8)	59 (9)	0.08	60 (13)	60 (13)	0.99
Oral contraceptive use, n (%)	538 (48)	704 (61)	<0.001	62 (39)	224 (45)	0.19	191 (52)	242 (59)	0.08
Number liveborn, n (%)			<0.001			0.09			
0	363 (32)	218 (19)		13 (8)	21 (4)		67 (18)	59 (14)	0.06
1	153 (14)	145 (13)		10 (6)	20 (4)		33 (9)	30 (7)	
2	280 (25)	341 (29)		45 (28)	133 (27)		92 (25)	107 (26)	
≥ 3	324 (29)	456 (39)		90 (57)	322 (65)		164 (45)	192 (47)	
Missing	0 (0)	0 (0)		0 (0)	0 (0)		8 (2)	24 (6)	
Tubal ligation*, n (%)	155 (14)	211 (18)	0.005	25 (16)	101 (20)	0.21			
Tumor histology, n (%)									
Serous	607 (54)			86 (54)			213 (59)		
Mucinous	140 (13)			18 (11)			28 (8)		
Endometrioid	165 (15)			16 (10)			64 (18)		
Clear Cell	139 (12)			5 (3)			20 (6)		
Other	69 (6)			33 (21)			38 (10)		
Tumor behavior, n(%)									
Invasive	848 (76)			134 (85)			310 (85)		
Borderline	269 (24)			22 (14)			54 (15)		
Missing	3 (<1)			2 (1)			0 (0)		

*Tubal ligation was not collected in the MAY study.

Table 2

Association between MTHFR polymorphisms and ovarian cancer risk in the New England Case Control Study (NEC), Nurses' Health Study (NHS), and Mayo Clinic Ovarian Cancer Case Control Study (MAY)

MTHFR SNP	Controls n (%)	Cases n (%)	MV Adjusted* OR (95% CI)	Pooled† OR (95% CI)
C677T (rs1801133)				
NEC				

MTHFR SNP	Controls n (%)	Cases n (%)	MV Adjusted* OR (95% CI)	Pooled† OR (95% CI)
CC	499 (49)	427 (40)	1.00	
CT	488 (43)	492 (46)	1.20 (0.99, 1.44)	
TT	138 (12)	140 (13)	1.22 (0.92, 1.60)	
p_{trend}			0.06	
NHS				
CC	210 (44)	71 (46)	1.00	1.00
CT	217 (45)	72 (47)	0.97 (0.65, 1.45)	1.16 (1.00, 1.35)
TT	55 (11)	10 (7)	0.60 (0.27, 1.30)	0.95 (0.64, 1.40)
p_{trend}			0.33	p_{trend} = 0.59
MAY				
CC	193 (47)	164 (45)	1.00	
CT	168 (41)	167 (46)	1.18 (0.87, 1.59)	
TT	51 (12)	33 (9)	0.83 (0.51, 1.37)	
p_{trend}			0.98	
A1298C (rs1801131)				
NEC				

MTHFR SNP	Controls n (%)	Cases n (%)	MV Adjusted* OR (95% CI)	Pooled† OR (95% CI)
AA	534 (49)	515 (50)	1.00	
AC	450 (41)	430 (41)	0.96 (0.80, 1.15)	
CC	109 (10)	93 (9)	0.84 (0.62, 1.15)	
p_{trend}			0.31	
NHS				
AA	236 (49)	68 (44)	1.00	1.00
AC	200 (41)	67 (44)	1.17 (0.78, 1.75)	0.97 (0.84, 1.12)
CC	48 (10)	18 (12)	1.39 (0.73, 2.65)	0.96 (0.75, 1.24)
p_{trend}			0.28	p_{trend} = 0.58
MAY				
AA	189 (46)	173 (48)	1.00	
AC	180 (44)	149 (41)	0.90 (0.66, 1.21)	
CC	43 (10)	42 (12)	1.05 (0.65, 1.69)	
p_{trend}			0.84	

*Multivariate models are adjusted for age, parity, oral contraceptive use, and tubal ligation. Multivariate models used in the MAY analysis did not include tubal ligation.

† For the C677T pooled estimate, the p for heterogeneity = 0.02. For the A1298 pooled estimate, the p for heterogeneity = 0.19.